NEUROSPORA. I. PRELIMINARY OBSERVATIONS OF THE CHROMOSOMES OF NEUROSPORA CRASSA [1]

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THE PRESENT report on the chromosomes of *Neurospora crassa* represents the results of observations which were confined to a period of ten weeks in the biological laboratories of Stanford University. The purpose of this study was to obtain some knowledge of nuclear and chromosome behavior in normal and mutant strains. The author realizes that no single phase of these investigations could be adequately studied in so short a period of time. Because of the interest in *Neurospora* as genetic material, a summary of some of these observations will be given at this time.

The observations were confined to the nuclei and chromosomes in the ascus, from fertilization to spore formation. Union of two haploid nuclei occurs in the young ascus. This is followed by a simultaneous enlargement of the ascus and fusion nucleus. During this growth period, the chromosomes in the fusion nucleus enter into meiotic prophase activities, including homologous association of chromosomes, elongation of chromosomes, chiasmata formation and contraction until typical metaphase I bivalents are produced. Although the consequences of this meiotic prophase activity are essentially similar to those observed in many other organisms, the timing of chromosome synapsis and elongation is dissimilar and is of some theoretical interest. The two meiotic mitoses follow in rapid succession leading to the formation of four haploid nuclei. In essential details and accomplishments, the chromosome and nuclear behavior in these two divisions is typical of meiosis in general. Particular details, however, are of interest to the cytologist. Each of the four haploid nuclei in the now greatly enlarged ascus undergoes a typical equational mitosis resulting in a row of eight haploid nuclei. Associated with each nucleus is a centriole which has become greatly enlarged during the meiotic and first post-meiotic mitoses. Fibers emerging from each centriole extend and encircle the cytoplasm surrounding each nucleus. This process initiates wall formation and the cutting out of eight independent ascospores. Shortly after the spore walls are differentiated, the nucleus in each spore undergoes an equational mitosis. The ascospore continues to maturity with the two resulting nuclei.

METHODS.—Approximately seven days (at 25° C.) after inoculation of an agar slant with the two sex strains, *A* and *a*, perithecia were present containing numerous asci in various stages of pre-fertilization, fertilization, meiosis and spore formation and development. These perithecia were removed from the slant and placed in a drop of staining solution. With the bent end of a needle, pressure was applied to the perithecial wall. When this pressure was properly exerted, the asci within the perithecium were forced out through the ostiole. They usually emerged as a single mass. The perithecial wall was removed and a cover slip placed over the drop. The slide was then gently heated. Several methods of staining were attempted such as aceto-orcein, aceto-carmine, propionic-orcein, lacto-orcein and acetic-lactic-orcein combinations. After many trials, it was realized that the genetic strain being utilized had much to do with the success of the staining procedure. A cross of two particular wild-type strains always gave excellent results, whereas other strains gave moderate or consistently poor results. In general, aceto-orcein was the most reliable chromosome stain but the nucleoli were not differentiated. When, in any particular aceto-orcein preparation, it was necessary to observe the nucleoli, aceto-carmine was subsequently run under the cover slip. The nucleoli, taking up the carmine stain, were then clearly visible.

CHROMOSOME NUMBER.—The haploid chromosome number in all the examined strains of *N. crassa* was seven. This number does not agree with that given by Lindegren and Rumann (1938) for *N. crassa* (six to nine chromosomes) nor that given by Colson (1934) for *N. tetrasperma* (six chromosomes). Seven haploid chromosomes had previously been observed (Dr. E. A. Weaver and author, unpublished) in a strain of *N. tetrasperma* supplied by Dr. B. O. Dodge. The author is indebted to Dr. G. W. Bohn, a former graduate student of the University of Missouri, for calling her attention to the *Neurospora* chromosomes. He observed seven haploid chromosomes in his excellent aceto-carmine preparations of *Neurospora sp.*

CHROMOSOME SIZE.—The lengths of the chromo-

[1] Received for publication August 28, 1945.

The author wishes to express appreciation to Dr. G. W. Beadle for presenting the opportunity for these studies and for the many kindnesses he has shown. Much credit should be given to Mrs. Mary B. Houlahan, Dr. Herschel K. Mitchell and Dr. Lotti Steinitz for their interest and collaboration in the chromosome studies, in selecting and supplying the mutant and wild-type strains and for various helpful suggestions regarding techniques.

somes were measured at various stages from presynapsis in the zygote nucleus to the metaphase of the division in the ascospore. The longest chromosome is approximately 2.7 times the length of the shortest chromosome. Since the relative lengths of all chromosomes are maintained throughout the nuclear cycles, measurements will be mentioned only for this longest chromosome. At the end of the pachytene, this chromosome may attain a length of approximately 15 microns. At metaphase of the third division in the ascus, it may be approximately 2.5 microns long. At the metaphase of the division in the ascospore, it may be only 1.5 microns long. The chromosomes of the hyphal nuclei were not examined. In contrast to the relatively large size of the nuclei in the ascus and the ascospores, the hyphal nuclei are very minute. It is probable that the metaphase chromosomes they form are likewise very minute.

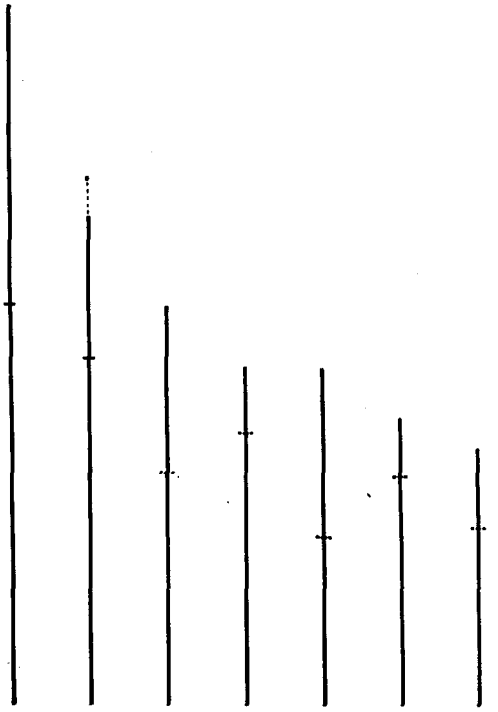

Fig. 1. Diagram illustrating the relative lengths of the seven chromosomes of *Neurospora crassa*. The cross lines indicate the positions of the centromeres; these are reasonably correct for the two longest chromosomes. The determination of the positions of the centromeres in the other chromosomes needs further confirmation; the assigned positions (broken cross-lines) should be considered only as tentative. The separation of the minute satellite from the main segment of the short arm of chromosome 2 is indicated by the dashed line.

RELATIVE LENGTHS OF THE CHROMOSOMES.—Measurements of the relative lengths of the chromosomes were most satisfactorily obtained from nuclei in late pachytene. The chromosomes are then at their maximum extension (see below). Although all seven chromosomes were drawn and measured in only a few meiotic prophase nuclei, the relative lengths of the chromosomes were consistent within each nucleus. Figure 1 illustrates the relative lengths of the seven chromosomes as computed from these measurements.

MORPHOLOGY OF THE CHROMOSOMES.—*Centromere positions.*—The centromere position was adequately determined only for the two longest chromosomes. The analysis of centromere positions was suspended temporarily because it was thought that one of the smaller pairs of chromosomes might be heteromorphic. If this were true, two sets of chromosome morphologies with respect to centromere positions, would have to be considered. The presence of a heteromorphic pair was not confirmed in subsequent examinations which were confined mainly to a cross between two particular wild-type strains. Whether a heteromorphic pair is present or could be identified in crosses of other wild-type strains remains to be determined. Due to the pressure for other determinations, no time was taken to renew the studies of centromere positions. In order to convey some idea of centromere positions in the complement as a whole, the tentative positions that had been assigned to chromosomes 3 to 7 before this analysis was suspended, are included in figure 1.

The nucleolus chromosome.—The second longest chromosome (chromosome 2) possesses a nucleolus organizer close to the end of its short arm. Consequently, there is a very minute satellite. The nucleolus organizer functions in the usual manner and develops a nucleolus in each telophase nucleus.

Chromomere patterns.—At late pachytene, each chromosome shows a distinct chromomere pattern. The pattern for any one chromosome is constant. The chromomeres have various sizes and shapes. They are separated by thinner strands of chromatin but are not spaced equally along the chromosome. The smaller chromosomes have only a few distinct chromomeres (five to six or seven), whereas the longer chromosomes have correspondingly more. No attempt was made in this preliminary study to map the chromomeres of each chromosome. However, these distinctive chromomere patterns could be useful in identifying individual chromosomes at pachytene. No knobs were recognized in these chromosomes. Centromeres could not be identified with certainty in the orcein stained preparations of pachytene.

Heterochromatin.—Heterochromatic segments of chromosomes were not recognized as such in the pachytene chromosomes. However, the presence of heterochromatin was detected in the telophase nuclei following the second meiotic mitosis and in the resting nuclei of the one- and two-nucleated ascospores. It could also be observed in the hyphal nuclei. There are two main segments of heterochromatin. They are located adjacent to a centromere. It has not been determined whether these two recognized segments lie adjacent to the centromere on opposite arms of one chromosome or whether they are parts of two separate chromosomes. Congression of the centromeres in late anaphase of division III,

and in the spore division, results in the formation of a somewhat pear-shaped resting nucleus. The centromeres of all seven chromosomes lie in the apex of this pear-shaped nucleus. Here, also, are found the two heterochromatic bodies lying so close together that they suggest a single dumb-bell shaped structure. It is believed, however, that they have not fused to form a single chromocenter but are forced close to one another by the intimate spacial association of all seven centromeres. Extensive observations have not been made of these two heterochromatic bodies nor has an attempt been made to identify the chromosome or chromosomes involved.

NUCLEAR FUSION, CHROMOSOME SYNAPSIS AND THE SUBSEQUENT ELONGATION OF THE SYNAPSED CHROMOSOMES.—Fusion of two haploid nuclei to form the zygote occurs in the very young ascus. Illustrations of the appearance of the ascus at this stage are given by Colson (1934). At the time of fusion, the chromosomes of each nucleus appear to be in a resting stage and a nucleolus is present in each. Following nuclear fusion, the chromosomes contributed by each nucleus undergo what appears to be a typical prophase contraction until, in some strains, the chromosomes may be almost as short as those of the metaphase of the third division in the ascus. No obvious doubleness of the chromosomes was observed, however. During this period, fusion usually occurs between the nucleoli contributed by each nucleus. At the end of the contraction period, the two haploid sets of chromosomes lie, roughly, at opposite sides of the zygote nucleus. In this highly contracted state, the homologous chromosomes enter into the synaptic phase of the meiotic cycle. In the early synaptic phase, many nuclei were observed with some homologous chromosomes lying adjacent to one another but not in actual physical contact. It is not clear whether this early stage in the association process is the consequence of a directed migration of homologues toward one another or whether this stage is reached following random movements of the chromosomes within the nucleus. Possibly the movements of the chromosomes could be followed in tissue cultures of the living asci. It is of considerable theoretical interest to determine the range of the force of synaptic attraction. The actual physical association of the chromosomes usually begins at one or both ends and continues along them. In many zygote nuclei, synapsis is completed for some pairs of chromosomes before the members of other pairs have come in contact. Soon, many nuclei show seven short, but completely synapsed, bivalent chromosomes. (Most of the detailed observations of the synaptic phase were confined to asci resulting from the cross of two wild-type strains (Emerson 5256*A* × Chilton-*a*). In crosses of some other strains, synapsis appears to occur when the chromosomes are less contracted.) Following completion of synapsis and possibly during this period, the chromosomes commence their elongation. This is possibly an uncoiling process for in some early postsynaptic nuclei, the elongating chromosomes appeared to possess compressed gyres. This elongation process continues until the chromosomes have reached their full extension. At this stage, the chromosomes are essentially similar in appearance to the pachytene chromosomes of many other organisms. The term "pachytene" has therefore been used. Although homologous chromosomes lie side by side at late pachytene, they are often not closely appressed. Often, there is little or no relational coiling of the two homologues around one another. During the period from zygote formation to late pachytene, the volume of the nucleus and nucleolus increases steadily. In all post-synaptic stages, the volume of the nucleus is very much greater than that of the chromosomes. Consequently, the chromosomes are widely spaced within the nucleus. During all these stages, the chromosome 2 bivalent remains attached to the nucleolus by the organizer regions. At pachytene, the organizer regions of the two homologues may diverge slightly from one another; the satellites may be some distance removed from them.

CHROMOSOME BEHAVIOR FROM DIPLOTENE TO THE THIRD DIVISION IN THE ASCUS.—At diplotene, a wide separation occurs between parts of a bivalent chromosome but the individual chromatids were difficult to follow. Coiling commences at diplotene and the contraction of the chromosomes is very rapid. At diakinesis, typical chiasmata may be seen. No attempt was made to count chiasmata but it is possible to do so at this stage. The chromosomes continue contraction to form typical metaphase I bivalents with terminal and interstitial chiasmata. Although the nucleolus becomes smaller during the pre-metaphase stage, it does not disappear. Chromosome 2 remains attached to the nucleolus by its organizer region. Anaphase I separation of the chromosomes appears to be essentially typical except for the nucleolus. This may be dragged toward one pole or stretched between the poles because the nucleolus organizers of one or both of the dyad chromatids of chromosome 2 have not been released from their attachment to the nucleolus. The nucleolus becomes detached before telophase sets in and may subsequently be seen in the cytoplasm of the ascus. At telophase I (and likewise telophase II and III) the centromere regions of all the chromosomes form an aggregate that lies at the apex of a distinct protrusion of the nucleus (the beak: Dodge, 1927). No true resting nucleus is formed. Instead, the chromosomes uncoil and the individual arms of each chromosome extend into an elongated nucleus. A new nucleolus is produced by and remains attached to the nucleolus organizers of chromosome 2. Prophase II proceeds by contraction of these elongated chromosomes until the two dyads of each chromosome form very short, parallel rods, each showing a conspicuous centromere region. Metaphase and anaphase II proceed normally. At telophase II, the chromosomes, whose centromere regions are again aggregated at the apex of the beaked nucleus, uncoil and the two arms of each chromosome extend into the nucleus as individual strands and remain in this con-

dition until the following prophase. The extent of elongation of the chromosomes appears to be similar to that of late pachytene. In each nucleus, a new nucleolus is formed at the position of the nucleolus organizer of chromosome 2. Prophase III proceeds by contraction of the arms of the chromosomes. Because the chromosomes maintain their previous telophase orientation (Js and Vs) during this contraction, the prophase of division III is a satisfactory stage for observing the relative lengths of the arms of a chromosome. Metaphase and anaphase of the

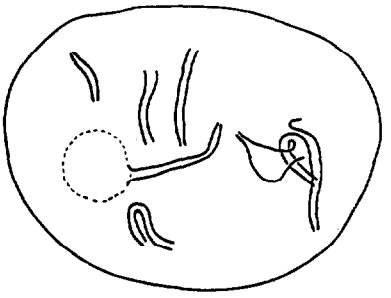

Fig. 2. Outline drawing of the synapsed chromosomes in an ascus heterozygous for T 4637. There are five bivalents and a synaptic configuration of four chromosomes. The nucleolus is outlined by a dashed line. The minute satellites of the pair of nucleolus chromosomes were not detected in this figure.

third division proceed as a typical equational mitosis. The telophase of this division is followed by a condition of the nucleus resembling a resting stage. Shortly after spore delimitation, a mitosis occurs in each ascospore. This is likewise a typical equational mitosis. In essential details, divisions I and II are typically meiotic. Division III is essentially a somatic mitosis except that the chromosomes retain their identity from the telophase of division II to the prophase of division III. It would be of interest to determine the time of effective splitting of the chromosomes for this division.

Reciprocal translocations.—In the Stanford laboratory, many mutants have been obtained following x-ray and ultra-violet irradiations. Chromosomal abnormalities could likewise be expected to occur from such treatments. Three irradiation-induced mutants (4637, 44105 and 45502) whose genetic behavior suggested the presence of some chromosomal abnormality, were selected and crossed to normal wild-type strains. The chromosomes were examined in the asci developing from these crosses. In all three cases, the ascus nuclei were heterozygous for a translocation between two non-homologous chromosomes. In the limited time available, it was not possible to make an intensive study of each translocation. Nevertheless, some observations and interpretations based on these studies will be mentioned.

Translocation 4637.—Figure 2 represents an outline drawing of late pachytene chromosomes in an ascus nucleus developing from the cross of the albino mutant strain 4637 by a wild-type strain. There are five normal bivalents and a synaptic configuration of four chromosomes (right). In these nuclei, homologous associations of all parts of the four chromosomes were not always accomplished. Unsynapsed segments, as illustrated in figure 2, were frequently observed. Sometimes, at pachytene, the four chromosomes were present as two "bivalents" with synaptic associations only between their respective homologous parts. At diakinesis and metaphase I, either a ring of four chromosomes, a chain of four chromosomes or two "bivalent" chromosomes were observed.

Translocation 44105.—Relatively few observations were made of the translocation introduced by mutant strain 44105. These were limited to a few figures of diakinesis and metaphase I. A ring of four chromosomes was observed in one metaphase I figure. In several others, one or more of the chromosomes were present as univalents. In two figures, all four chromosomes were present as univalents. No pachytene configurations were observed.

Translocation 45502.—The reciprocal translocation introduced by mutant strain 45502 involved a very unequal exchange of segments of two non-homologous chromosomes. The breaks appear to have occurred close to the end of the long arm of chromosome 1 and close to the centromere in the long arm of one of the chromosomes with a sub-terminal centromere. This translocation could serve several purposes which will be outlined below.

Estimates of the types of disjunction of chromosomes in asci heterozygous for T 45502.—Because of the small size of the metaphase and anaphase I chromosomes in *Neurospora,* it would be very laborious to determine by direct observations the modes of disjunction of the four chromosomes involved in translocation configurations. An examination of the eight-spored asci developing from asci whose fusion nuclei were heterozygous for translocation 45502 has suggested a possible method of estimating these disjunctions. In most organisms, a two-by-two disjunction of the four chromosomes of an interchange complex usually occurs at anaphase I. In organisms having the *Oenothera* type of disjunction, alternate chromosomes in a ring or chain of four or more chromosomes go to the same pole at anaphase I. In maize, *Pisum,* etc., the four chromosomes of a ring usually disjoin so that two members go to one pole and two to the opposite pole. In these forms, alternate disjunctions occur in some cells. In other cells, however, two adjacent members of the ring or chain of four chromosomes may go to the same pole. When a heterozygous translocation is present in *Neurospora,* do the chromosomes disjoin according to the *Oenothera* pattern or do disjunctions follow the maize and *Pisum* pattern? The analysis given below suggests that the disjunctions in *Neurospora* are similar to those observed in maize and *Pisum.*

Although the exact position of breakage in the two chromosomes has not been determined, a diagram illustrating the type of synaptic configuration to be expected in asci heterozygous for T 45502 is given in figure 3. If no crossing over occurs in either re-

gion *a* or *b*, figure 3, alternate disjunctions (1+4 : 2+3, fig. 3) of the four chromosomes at anaphase I when a ring or a chain is present, or the counterpart type of disjunction when two "bivalents" are present, should produce an ascus with eight normal spores (Type I ascus, fig. 3). In this case every spore would receive a full genomic complement, four with the normal chromosomes (1+4, fig. 3), and four with the translocation chromosomes (2+3, fig. 3). When two adjacent chromosomes of this complex pass to the same pole at anaphase I, all eight of the resulting spores in an ascus would be deficient for some part of the genomic complement. There are two possible types of adjacent disjunctions, those which result from disjunctions of homologous centromeres (1+2 : 3+4) and those which result from non-disjunction of homologous centromeres (1+3 : 2+4). The former will be called adjacent I disjunction, the latter, adjacent II disjunction. Following adjacent I disjunctions, four of the spores (with 1+2) would be deficient for nearly all of the long arm of one chromosome. In contrast, the four spores with 3+4 would be deficient only for a small segment of the genomic complement. Comparable studies in maize have shown that spores with deficiencies of large segments of the genomic complement are defective in appearance, whereas spores with small deficiencies may be normal in appearance, especially in the early developmental stages. If the response in *Neurospora* is similar, it could be expected that the spores with 1+2 would be defective in appearance, whereas those with 3+4 may be normal in appearance, especially in the young eight-spored asci. If this occurs, adjacent I disjunctions would give rise to asci with four adjacent defective spores and four adjacent spores which appear to be normal (Type III ascus, fig. 3). If adjacent II disjunctions occurred, all eight spores would be deficient for relatively large segments of the genomic complement. All eight spores could be expected to show visible evidence of the deficiencies (Type IV ascus, fig. 3).

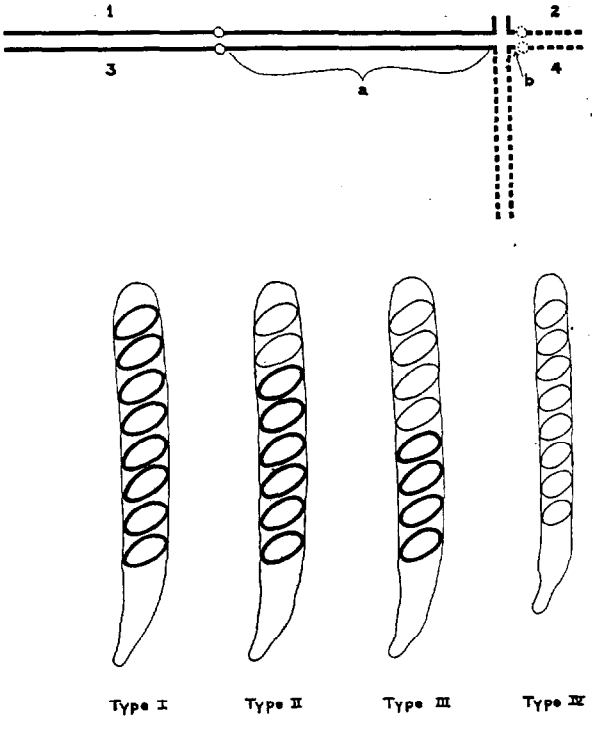

Fig. 3. *Upper.* Diagram illustrating the complete synaptic association of two normal and two translocated chromosomes in an ascus heterozygous for a very unequal reciprocal translocation. Chromosomes numbered 1 and 4 represent the normal chromosomes; chromosomes numbered 2 and 3 represent the translocation chromosomes. The centromere in each chromosome is represented by a circle. *Lower.* Diagrammatic representation of the types of eight-spored asci resulting from several types of disjunctions of the four members of the synaptic complex (see text for explanations). The heavily outlined spores are normal in appearance; the lightly outlined spores are visibly defective in appearance. (In the observed material, the type IV ascus is considerably more defective than the diagram suggests.)

On the supposition that the spores with 1+2 are defective in appearance and those with 3+4 are normal in appearance, a fourth type of eight-spored ascus could be anticipated. This would be formed whenever a crossover had occurred between the centromere and the position of break (regions *a* or *b*, fig. 3). In this *Neurospora* translocation, such a crossover is probably confined almost entirely to the long segment of region *a*. Few crossovers would be expected to occur in the very short *b* segment. Studies of the disjunction of the four chromosomes involved in a heterozygous translocation in maize have revealed that whenever a crossover has taken place between the centromere and the position of breakage, homologous centromeres will pass to opposite poles at anaphase I (McClintock, unpublished). If this crossover-disjunction relationship likewise applies to *Neurospora*, the resulting eight-spored asci should possess four spores with normal genomic complements (two with 1+4 and two with 2+3), two normal appearing spores with the short deficiency (3+4) and two adjacent defective spores with the longer deficiency (1+2) (Type II ascus, fig. 3).

As table 1 indicates, four main types of asci corresponding to types I to IV, figure 3, were observed. The eight-spored asci were all relatively young, as the counts were made from slides prepared for chromosome studies. In each count, the relative frequencies of the types of asci are similar. Observations of the spore appearances in mature asci were made by Mrs. Mary B. Houlahan. She found that the asci with two very defective spores had, in addition, two immature appearing spores plus four normal appearing spores. These should be type II asci; the spores with the short deficiency, (3+4), not distinguishable in the young stage from spores having a normal genomic complement, are now detectable because of their slower rate of maturity.

It should be stated that in ascus type II, the two adjacent defective spores occupied any one of the four possible positions in the ascus, with approxi-

TABLE 1. *Frequencies of asci with normal and defective spores in six preparations. The zygote nuclei were heterozygous for a translocation associated with mutant strain 45502.*[a]

Type I ascus All 8 spores normal	Type II ascus 2 defective sister spores and 6 normal spores	Type III ascus 4 adjacent defective spores and 4 normal spores	Type IV ascus All 8 spores defective
20	50	28	17
24	41	34	8
37	85	32	18
37	58	30	17
54	97	50	16
25	50	24	17
Totals			
197	381	198	93[b]

[a] Record was made of 17 asci with normal and defective spore orientations other than types II and III of this table. See text for description.

[b] In making these slides for chromosome studies, many of the asci of types I to III were broken and their spores scattered. Only non-broken asci were scored. Type IV asci were not so readily broken. Thus, the figure for type IV probably is relatively too high.

mately equal frequencies. This is to be expected if the orientation of the chromosomes at metaphase I and II is at random with respect to the long axis of the ascus. Likewise, in ascus type III, the four adjacent defective spores occupied positions either at the base or the tip of the ascus.

On the basis of the explanation of the types of eight-spored asci given above, the following conclusions may be drawn: (1) When no crossing over occurs between the centromere and the point of interchange, alternate and adjacent I disjunctions will occur equally frequently (types I and III, table 1). (2) Adjacent II disjunctions are relatively infrequent (type IV, table 1; see accompanying footnote). (3) A crossover occurs in the longer chromosome between the centromere and the position of breakage in approximately half of the ascus nuclei (type II, table 1). It is fully realized that these studies are only preliminary and require further investigation. Nevertheless, the author wishes to emphasize the possible usefulness of this type of analysis as a complement to the cytological observations.

A POSSIBLE METHOD FOR DETERMINING THE FREQUENCY OF TRANSPOSITION OF SPORES.—In many genetic analyses, the order of the spores in an ascus is of prime importance. The eight spores in an ascus are linearly arranged and are assumed to reflect the orientation of the nuclei and spindles in the three preceding divisions in the ascus. Following division I, the two resulting nuclei are some distance apart in the ascus cytoplasm. The spindles they form are parallel to the long axis of the ascus. Thus, following the second division, four nuclei are present, the upper two derived from one nucleus, the lower two derived from the second nucleus. Maintaining their respective positions in the ascus cytoplasm, each nucleus again divides and a row of eight free nuclei are formed. It is not until then that walls appear cutting out the eight spores. If no disturbances have occurred in the arrangement of the nuclei and spindles during the free-nucleated stage, the position of each spore reflects its origin with respect to the three preceding divisions. Lack of wall formation following divisions I and II in the ascus is a distinct disadvantage. Irregularities in spindle orientation or transposition of the usual order of two or more of the free nuclei will lead to linear arrangements of spores which do not reflect their origin in the previous spindles. Irregularities of this sort are known to occur and it is important for some investigations to determine their frequencies.

The reciprocal translocation in mutant strain 45502 or a chromosomal abnormality giving similar types of recognizable defective spores, might be useful for estimating the frequency of occurrence of aberrant alignments of some of the spores in an ascus. In addition to the ascus types recorded in table 1, there were 17 asci with normal and defective spore orientation other than types II and III. If, after the second meiotic mitosis following an adjacent I disjunction described above, the two inner nuclei (with 1+2 and 3+4, respectively) exchanged positions, the spore alignment would not be type III. Instead, two adjacent normal appearing spores (with 3+4) would be inserted between the two sets of sister defective spores (with 1+2). Seven of the 17 aberrant asci were of this type. If, following division III in an ascus destined to be of type II, two non-sister nuclei exchanged positions, a spore alignment other than type II could appear. This would occur if one of these nuclei possessed the long deficiency (1+2) which gives rise to the defective appearing spores. In these asci, the two defective appearing spores would now be separated by a normal appearing spore. Five such asci were observed among the 17 aberrant asci mentioned in the footnote to table 1. These observations are not considered adequate for estimating the frequency of nuclear displacements. More study needs to be given to the aberrant asci to determine whether displacement of spores may occur after spore delimitation through rough handling, or whether additional disturbances, such as aberrant chromosomal behavior, are contributing factors. Because of the significance of aberrant alignment of spores in genetic investigations, it was considered worth while to mention a possible rapid method of estimating their frequencies.

CONCLUSIONS.—The usefulness of fungi as genetic material has been well demonstrated in recent years. To interpret properly the results of many genetic investigations, it is either advantageous or necessary to know the accompanying chromosomal conditions. On the basis of this brief study of *Neurospora* chromosomes, the author anticipates that some fungi may prove to be adequate and in some respects superior cytogenetic material. A review of the literature sug-

gests that some forms may be distinctly superior to *Neurospora* for studies of chromosome behavior, particularly of those stages from fertilization to the first meiotic metaphase. Forms with two haploid chromosomes, one of which is associated with the nucleolus, might prove to be very satisfactory in following the stages and motions of the chromosomes during synapsis, in studying the consequences of various chromosomal rearrangements and for other studies involving the meiotic prophase periods. In ascomycetes, the ease of isolation of the asci, the abundance of asci and the relation of size to stage in meiosis should recommend this material for tissue cultures when it is desired to observe the chromosomes during the meiotic stages in living nuclei.

The haploid chromosomal complement of *Neurospora crassa* is similar in its organization to that observed in many organisms. Each of the seven chromosomes may be identified not only by its relative length, the position of its centromere, but also by the constancy of its internal organization as exhibited by chromomere patterns in the meiotic prophase. One chromosome of the haploid complement possesses a nucleolus organizer which functions just as it does in other organisms. Because of the location of the nucleolus organizer near the end of one arm of this chromosome, there is a minute satellite. Even the coiling and uncoiling processes leading to contraction and expansion of the chromonema appear to be similar to that observed in many other organisms. No distinctively unique features of chromosomal organization were recognized. The presence of translocations between non-homologous chromosomes following irradiation treatment and the behavior of these translocated chromosomes in the meiotic stages of heterozygous asci likewise are indicative of the orthodox organization of the *Neurospora* nuclei and chromosomes.

It has been observed that the behavior of the chromosomes in the first two mitoses in the ascus results in the formation of four haploid nuclei whose chromosomes have been subjected to the processes common to meiosis in general: synapsis of homologous chromosomes, chiasma formation, and typical anaphase I and II disjunctions and segregations of chromatids. The synaptic period, however, is distinctly atypical. In many organisms, synapsis is initiated in the meiotic prophase when the chromosomes are much extended. In the *Neurospora* strains most extensively studied, this period occurs when the chromosomes are contracted, short rods simulating late prophase chromosomes. Elongation of the chromosomes to their maximum meiotic prophase extension takes place after the chromosomes have become homologously associated throughout their lengths. If the chromonema within each chromosome at the time of synaptic attraction and association is tightly coiled, the homologous associations along the chromosomes cannot be equally intimate. Other cases of synaptic attraction of condensed chromosomes have been described but *Neurospora* offers rather unique opportunities for studying this process.

The centriole has not been considered in previous sections of this report, but it deserves a brief mention because of its steady enlargement during the interphase stages of the divisions in the ascus, its relation to the centromeres during this enlargement, as well as its previously known function in initiating spore wall formation (Harper, 1905; Dodge, 1927; Wilcox, 1928). As mentioned previously, the interkinetic nuclei following divisions I, II and III are somewhat pear-shaped because of a decided protrusion or "beak." The centromeres of all chromosomes form a compact aggregate at the apex of this beak. The centriole begins to enlarge into a rod-shaped structure following division I. It functions as a typical centriole in division II. During the following interkineses, the process of enlargement in contact with the centromeres continues. It again functions as a typical centriole during the third mitosis. (For illustrations, see Plates I and II, Dodge, 1927). Following the third division, the greatly elongated centriole, associated with the beak of each nucleus, comes to lie close to the ascus wall. Fibers emerge from it and encompass a mass of cytoplasm about each nucleus thus initiating spore wall formation. That centromeres, centrioles and blepharoplasts are interchangeable cell organelles has been demonstrated in the classic investigations of Pollister and Pollister (1943). In line with these investigations, it is possible to consider that the centromeres of *Neurospora* may contribute to the substance of the centriole during these periods of enlargement. Centromeres, centrioles and blepharoplasts all have the common function of producing fibers. It is possible that the fibers formed by these three interrelated but morphologically distinct cellular organelles are structurally identical or much alike in that they all possess one particular type of molecular organization which is responsible for their capacity to contract or alternately contract and expand.

SUMMARY

A summary report is given of the results obtained from a very brief study of chromosome and nuclear behavior in *Neurospora crassa*. The investigations are admittedly incomplete and possibly some errors have been made. Nevertheless, they have revealed that *Neurospora* offers adequate and in some respects unique opportunities for cytogenetic research. The chromosomes were followed from the nuclear division preceding zygote formation through the division in the ascospore. Chromosome morphology was considered with reference to the absolute and relative sizes of the seven chromosomes in various division cycles, the centromere positions, the nucleolus chromosomes, the pachytene chromomere morphology and the presence of heterochromatin. Chromosome behavior was followed with reference to the atypical timing of chromosome synapsis, the elongation of the chromosomes during a prolonged "pachytene," chiasma formation and the general behavior of the chromosomes in the two meiotic mitoses and the two subsequent equational mitoses. Several re-

ciprocal translocations were investigated and their usefulness for special studies indicated.

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